

NOV-16-2004 01:40PM FROM-Gen-Probe Incorporated - Patent Dept. +1 858 410 8928 T-375 P.014/041 F-136

EXHIBIT A

TITLE M. tuberculosis's 18S region T₇ primer /From Page No. - run 42°C / 10% glycerol / 10% DMSO / 5% m₁b rRNA

Materials -

X-alkaline gel/15mm	\rightarrow T ₇ A.mtb B (-) 290-3' R. P. 2118-6A	8.400/mg 0.8mL/15mmal
Mtb (+) 237	2082-79	3.200/mg 1.7mL/15mmal
9x GP/mtb	2120-98	RT / 733.50, 1200U/min
(10X) KNTP	2120-96	T ₇ A.mtb 303, 400U/15mL
10mm dNTP	2090-81	500mM TMAc 2120-95
NalC - 50G	2120-97	(tetramethyl ammonium)al
New primer T ₇ A.mtb A (-) 236	1802-71	1.99.00/mg, 53mL, 37pmol, 4.1m
T ₇ A.mtb A (-) 237	1802-3	1.59.00/mg, 47mL, 34.2pmol, 4.4m
X-alkaline 3ml/15mm	\rightarrow T ₇ A.mtb A (-) 251	12.12.00/mg, 53mL, 23.7pmol, 0.63m
X-alkaline 2.3ml/15mm	MgaA (+) 146,	2082-62, 11.00/mg, 31mL, 38.5pmol, 0.45m

(I) Design. Primer set

$$\text{T}_7\text{A.mtb B} \rightarrow 290-3' \text{R.P.} / \text{G} \rightarrow 237 \text{R.A.} = \textcircled{2} \quad 5 \text{fg}/\text{10mL} \quad \text{4.8mL}$$

$$\begin{aligned} \textcircled{1} \text{S} \left(\text{2.3mL} \text{mgoA} (+) 146 \right) / \text{T}_7\text{A.mtb B} \rightarrow 246-14.1mL & \text{ } \textcircled{1} - \textcircled{2} \\ / \text{T}_7\text{A.mtb A} \rightarrow 247-14.1mL & \text{ } \textcircled{3} - \textcircled{18} \\ / \text{T}_7\text{A.mtb A} \rightarrow 251-25.6 \text{mL} & \text{ } \textcircled{9} - \textcircled{24} \\ / \text{T}_7\text{A.mtb A} \rightarrow 251-13.2mL & \text{ } \textcircled{25}, \textcircled{26} \end{aligned}$$

0.5g/10mL (0.2% BSA) 4.5mL

(II) Mix

1x 280mL

(IV) HPA:-

9x GP/mtb	11	308	lyophilized probe mix (-) 27
10X KNTP	10	280	100mL Helper and Helper
10mm dNTP	10	280	probe m ₁ b A (+) 190 Rxn (78.3%)
Glycerol	10	280	helper m ₁ b A (+) 175 1.79%mg
DMSO	10	280	helper m ₁ b A (+) 219 1.3500/m
500mM TMAc	2	5.6	
0.5 phenol	0.5	1.4	aliquot 6.5mL x 26
rad H ₂ O	13	364mL	

(III) m₁b rRNA from L₁: a) 10fL/10mL (dil from L₁ 1:45)i) 2mL + 99.8mL H₂O (20amol/mg) \rightarrow stored -70°C probe mix (x30)ii) 2mL + 99.8mL H₂O total 9mL x 30 = 270mLiii) 2mL + 264mL (-) 0.2% BSA \rightarrow 5.5g/10mL

D. Hgb Rgt2 (15mM/1mL) 50mL x 30 (500mL)

(2) (-) 50mL x 30 (500mL)

probe 50fmol 7.4mL

(3) helper + 75.25pmol 1.93mL

addition (4) helper + 219.25pmol 9.14mL

(5) H₂O Q104.00mL 182.00mL

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

L. Wang

Result:

LEADER I *[Signature]* SER. # 1111
 RAW DATA SERIES MEASUREMENTS
 V. 3.5

MEAS. TIME(s) : 2.8 M+bA(r)190

SAMPLE	RLU	OFg
1	97882	$\times 10^{-5}$ total counts

MEAS. TIME(s) : 2.8 *T_{TA}Am+bA(r)251*

SAMPLE	RLU	OFg
1	469198	$\times 10^{-5}$ /10 ⁻⁵ 347.1/10 ⁻⁵
2	42265	1000 T _{TA} A
3	1597326	0.5A(r)
4	45053	
5	45675	
6	1443728	

MEAS. TIME(s) : 2.8 *425C x 1 hr 20'*

SAMPLE	RLU	OFg
1	2155	$\times 10^{-5}$ M+bA(r)190
2	2173	$T_{TA}Am+bA(r)251$
3	559	$\times 10^{-5}$ 1000 T _{TA} A
4	439601	0.5A(r)
5	448655	
6	427838	$T_{TA}Am+bA(r)246$
7	431805	$\times 10^{-5}$ 1000 T _{TA} A
8	437224	0.5A(r)
9	425262	
10	437259	
11	418198	$T_{TA}Am+bA(r)247$
12	421466	
13	424898	
14	401789	
15	419483	
16	439176	$T_{TA}Am+bA(r)251$
17	433466	
18	441665	
19	427486	
20	432622	
21	429669	

T_{TA} → 246, T_{TA} → 247 & T_{TA}Am+bA(r)251
looks very good.